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The plant hormone auxin plays an instrumental role in most plant developmental processes. It has been proposed that the complexity of the auxin signal transduction pathway might be responsible for the diversity of auxin effects. Auxin signal transduction is under the control of the Aux/IAA and ARF families of transcription regulators. There are 29 *Aux/IAA* genes that encode mostly short-lived repressors of auxin-inducible genes. The 23 ARFs can be either activators (the so-called Q-rich ARFs) or repressors of transcription and it has been shown that auxin transduction likely functions through dimerization of Aux/IAAs with ARFs. These complexes bind to Auxin-Response Elements (AuxRE) in the promoter of auxin-inducible genes, thus preventing transcription. By promoting the degradation of Aux/IAAs, auxin would allow the ARF activators to activate transcription. However only a very limited number of Aux/IAAs and ARFs have been tested and the role of the ARF repressors is largely unknown. In order to obtain a full picture of the auxin signaling pathway and understand further its function, we have used a yeast two-hydrid (Y2H) approach to test all the possible 1250 interactions between the 23 ARFs and 29 Aux/IAAs.

Exploring the auxin signaling network topology

Since the auxin signaling network shows complex topological features (with 433 non-oriented edges), clustering methods may be useful for summarizing the network topology into a small number of relevant classes. Here, we applied a recently developed model-based strategy that aims at clustering vertices based on their connectivity profiles (Daudin *et al.*, 2008; Picard *et al.*, 2009). The similar connectivity profile assumption is particularly adapted to the auxin signaling network since this profile for a given Aux/IAA or ARF is likely to be the basis for its biological function. The proposed model is a mixture model for random graphs, also referred to as the block clustering model. This model is implemented in the MixeR R package which is freely available. The block clustering model has proved to be more general that more classical graph clustering methods (Schaeffer, 2007) that aim at finding groups of vertices that are highly intra-connected and poorly inter-connected. Instead of directly describing the clustered structure of vertices, the block clustering model describes the topology of the network using connectivity probabilities π_{ql} i.e. the probability for a vertex from class *q* to be connected with a vertex from class *l*.

Once the mixture model for graph has been estimated, it is of interest to assess the adequacy of the clustering e.g. the separability of the clusters, the dispersion of the elements within the clusters. Since the assignment of elements to clusters is almost deterministic, this assignment can be viewed as a partition. We propose to use the edges incident to the vertices to derive dissimilarity measures for the vertices using the adjacency information. The Sokal-Michener distance between vertices *i* and *j* can be defined as the proportion of mismatches between the *i*th and *j*th rows of the adjacency matrix from which the within- and between-cluster distances can be directly defined. This distance naturally expresses the difference in connectivity profiles between vertices.

The results are presented for 3 clusters. The proteins are ordered from the most to the least central in the cluster in terms of average distance to other proteins of the cluster. The average distance is given for the most central, the most peripheral protein and some other proteins of interest for interpretation.

The proteins in italics are grouped to form a fourth cluster in the case of 4 clusters. The proteins in boldface are expressed in the shoot apical meristem.
cluster 1 (22 proteins): IAA3 (0.22), **IAA8**, **IAA18**, IAA2, IAA4, IAA1, **IAA16**, IAA10, **IAA13**, IAA28, IAA15, **IAA12**, **IAA27**, **IAA19,** IAA14, IAA17, **IAA20**, **IAA30**, IAA7 (0.33) | *IAA31* (0.37), *IAA33*, *IAA32* (0.44).
cluster 2 (9 proteins): **ARF5** (0.18), **ARF19**, **ARF8**, **ARF7**, **ARF6** (0.24), IAA5 (0.28), **ARF9**, **IAA9**, IAA34 (0.31);
cluster 3 (18 proteins): **ARF11** (0.10), ARF14, **ARF3**, **ARF1**, ARF13, IAA6, **ARF4**, **ARF18**, ARF16, ARF17, **ARF10**, **ARF2**, ARF12, ARF20 (0.20) | *ARF22* (0.23), *IAA11*, ***IAA29***, ***IAA26*** (0.26);
The connectivity probability matrix is

$$\Pi = \begin{pmatrix} 0.68 & 0.87 & 0.11 \\ 0.87 & 0.34 & 0.21 \\ 0.11 & 0.21 & 0.04 \end{pmatrix}.$$

Cluster 1 corresponds to most of the Aux/IAAs. The core of the cluster 2 corresponds to the ARF activators (ARF5, 6, 7, 8 and 19, the five most central elements within the cluster). Cluster 3 corresponds mainly to the ARF repressors (the rest of the ARFs), which are sparsely connected; see the third row of matrix Π. In the case of 4 clusters, the 3 most peripheral proteins of cluster 1 (which are three auxin non-degradable Aux/IAAs) are grouped with the 4 most peripheral proteins of cluster 3 (comprising three Aux/IAAs, two auxin-induced among them) to form a second Aux/IAA cluster. If the clustering approach is applied to the subset of proteins expressed in the shoot apical meristem, the 3 clusters obtained are almost nested in the three clusters shown above, the only exceptions being IAA29 and IAA26, relatively peripheral in cluster 3, which are then assigned to cluster 1 with almost all the others Aux/IAAs.

Using network topology to model auxin signaling

An increase in auxin level induces a rapid reduction of Aux/IAA levels, and thus of gene repression. Auxin was thus proposed to activate genes by de-repression. This system is built around a negative feedback loop, since most Aux/IAA proteins are encoded by genes which are themselves targets of the auxin signaling pathway.
Relying on the clustering analysis of the network structure, we developed an ordinary differential equation model of the dynamics of this pathway. The previous analysis suggests that the global behavior of the system can be accounted for by using only three types of elements : Aux/IAA proteins, ARF activators and repressors. Moreover, since the ARF repressors present few interactions with the other proteins, they were considered as a background level of transcriptional repression, taking the form of a scalar parameter β_A^-. Similarly, a parameter β_A^+ accounted for the positive ARF level.
For these abstract variables, the reaction diagram of the system is shown in Figure 1, where the reaction rates (we assume mass action law kinetics) should be interpreted as apparent kinetic rates, at the level of the full protein families. The auxin responsive genes were considered in this model as a single variable, which reflects the probabilities of being in one of the four states pictured in Fig. 1 : free, bound to a single ARF, or bound to an ARF:Aux/IAA or ARF:ARF dimer. Only the ARF or ARF:ARF bound situations were assumed to lead to transcription. Moreover, the Aux/IAA proteins were assumed to be coded by some auxin responsive genes, leading to the occurrence of the negative feedback loop in the system mentioned above. Auxin was described as an input to the system, by means of a parameter *aux* such that Aux/IAA decay rates increase with *aux*, as shown in Figure 1.

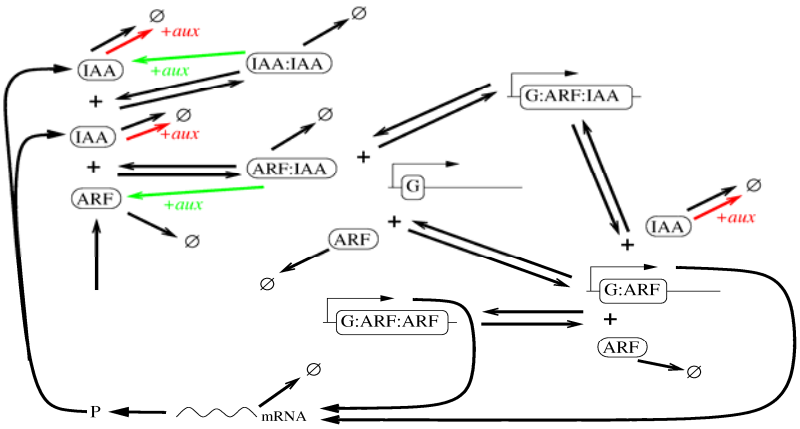

Figure 1. Reaction network. ARF and Aux/IAA (IAA above) can form dimers in a reversible way. A pool of auxin responsive genes is denoted by G, to which ARF bearing complexes can bind, via two pathways for ARF:IAA. Arrows represent the possible transformations between elements, and + represent binding of proteins and/or promoter of the G pool of genes. Degradation is represented by →Ø.

We then analyzed the type of behavior predicted by this model. To this aim, we fixed the order of magnitude of as many parameters as we found in the literature, which includes in particular decay rates of ARF and Aux/IAAs, as well as mRNA of the latter (Dreher et al. 2006). We were able to show that the system always presents a unique equilibrium state, which is perturbed by changes in auxin level. We then changed one by one the parameters of the model to observe their influence. In particular, we studied how these parameters affect the response of the system to variations of the auxin level. Actually, this data shows that ARFs (of both type) are essentially absent from the center of the meristem, and expressed at the periphery. Hence, increasing the levels ARF_A^- and ARF_A^+ from zero to higher values can be interpreted as considering a radial section of the meristem, with low levels representing the central zone (CZ), and higher levels the peripheral zone (PZ).
In conclusion, the absence of ARF activators in the center makes this region auxin-insensitive, in contrast with the PZ, where auxin induces transcription of the target genes as soon as some ARF activators are expressed. Hence, this patterning of ARFs and Aux/IAAs implies that the appearance of new primordia upon auxin induction may only occur in the PZ. Moreover, one prediction of the model is that for large ranges of reasonable parameters, the effect of ARF repressors is to reduce globally the response to auxin, and in particular the sensitivity to auxin variations. Hence, this model suggests that a potential role for these ARFs would be that of a buffering effect, allowing to reduce the effect of transitory auxin fluctuations in the PZ, and favor more stable signals, at the loci of emerging primordia.

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